

WHAT IS CLAIMED IS:

1. A method by which a sample is assayed to determine the presence or level therein of a first analyte relative to a second analyte, the method comprising the step of bringing the sample into contact with a labeling means adapted to label the first analyte through a binding interaction that is inhibited by the second analyte.
2. A method according to claim 1, wherein the labeling means comprises a conjugate having a component for binding the first analyte, and a component which binds antibody to the second analyte.
3. A method according to claim 2, wherein the labeling means comprises a second analyte and antibody to the first analyte.
4. A method according to claim 2, wherein the labeling means further comprises antibody to the second analyte having a detectable label bound thereto.
5. A method for identifying in a sample the presence or level of a preselected analyte originating from a target source, wherein any level of said preselected analyte in said sample originating from a source other than said target source is associated with a level in said sample of a marker from said source other than said target source, the method comprising conducting an assay following the sequential steps of
 - (a) first contacting the sample with an analyte labeling reagent comprising

- (1) a mobile, labeled binding partner to one of the analyte or the marker,
- (2) a conjugate between the marker and one of biotin and streptavidin, and
- (3) binding partner to the preselected analyte conjugated to the other of biotin and streptavidin; and then

- 5 (b) contacting the sample with an immobilized binding partner to the other of the preselected analyte and the marker;

wherein the extent of labeling of the immobilized binding partner is indicative of the presence or level of the preselected analyte in the sample reduced by the level of the marker in the sample originating from the source other than the target source .

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6. The method according to claim 5, comprising the sequential steps of:

- (a) first contacting said sample with a mobile, labeled binding partner to said marker,
- (b) next contacting said sample with a conjugate between said marker and a binding partner to said preselected analyte; and

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- (c) next contacting the sample with an immobilized binding partner to said preselected analyte.

7. The method of claim 5 wherein said binding partners are antibodies.

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8. The method of claim 5 wherein said sample is a biological sample.

9. The method of claim 8 wherein said sample is whole blood, serum, plasma, or urine.

10. The method of claim 5 wherein said preselected analyte is a cardiac analyte.

11. The method of claim 10 wherein said cardiac analyte is myoglobin, and said marker is carbonic anhydrase III.

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12. The method of claim 5 wherein said label is colloidal gold.

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13. The method of claim 6 wherein said mobile, labeled binding partner to said marker is a gold-labeled monoclonal anti-carbonic anhydrase III antibody, said conjugate between said marker and a binding partner to said preselected analyte is a conjugate between carbonic anhydrase II and an anti-myoglobin monoclonal antibody, and said immobilized binding partner to said preselected analyte is an anti-myoglobin monoclonal antibody.

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14. The method of claim 6 wherein said conjugate between said marker and a binding partner to said preselected analyte comprises a single-chain polypeptide.

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15. A method for identifying in a sample the presence or level of a preselected analyte originating from a target source, wherein any level of said preselected analyte in said sample originating from a source other than said target source is associated with a level in said sample of a marker from said source other than said target source, the method comprising conducting an assay following the sequential steps of

(a) first contacting the sample with an analyte labeling reagent comprising

(1) a mobile, labeled binding partner to the marker,

(2) a conjugate between the marker and one of biotin and streptavidin, and

(3) a binding partner to the preselected analyte conjugated to the other of biotin and streptavidin; and then

(b) contacting the sample with an immobilized binding partner to the preselected analyte;

wherein the extent of labeling of the immobilized binding partner is indicative of the presence or level of the preselected analyte in the sample reduced by the level of the marker in the sample originating from the source other than the target source .

10 16. A method according to claim 15, comprising the sequential steps of

(a) first contacting said sample with a mobile, labeled binding partner to said marker,

(b) next contacting said sample with a conjugate between said marker and streptavidin;

15 (c) next contacting said sample with a biotinylated binding partner to said preselected analyte; and

(d) next contacting the sample with an immobilized binding partner to said preselected analyte.

17. The method of claim 16 wherein said binding partners are antibodies.

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18. The method of claim 16 wherein said sample is a biological sample.

19. The method of claim 18 wherein said sample is whole blood, serum, plasma, or urine.

20. The method of claim 15 wherein said preselected analyte is a cardiac analyte.

21. The method of claim 20 wherein said cardiac analyte is myoglobin, and said marker is
5 carbonic anhydrase III.

22. The method of claim 16 wherein said label is colloidal gold.

23. The method of claim 16 wherein said mobile, labeled binding partner to said marker is a
10 gold-labeled monoclonal anti-carbonic anhydrase III antibody, said conjugate between
said marker and streptavidin is a conjugate of carbonic anhydrase III and streptavidin,
said biotinylated binding partner to said preselected analyte is biotinylated anti-
myoglobin monoclonal antibody, and said immobilized binding partner to said
preselected analyte is an anti-myoglobin monoclonal antibody.

15 24. The method of claim 16 wherein said conjugate between said marker and streptavidin is a
single-chain polypeptide.

20 25. A conjugate comprising an analyte or fragment thereof and streptavidin or a biotin-
binding fragment or variant thereof, wherein independently, said analyte or fragment
thereof in said conjugate is capable of being bound by an antibody to said analyte, and
said streptavidin or biotin-binding fragment thereof in said conjugate is capable of
binding to biotin.

26. The conjugate of claim 25 wherein said analyte or fragment thereof is a protein or peptide.
- 5 27. The conjugate of claim 26 wherein said analyte or fragment thereof and said streptavidin or biotin-binding fragment thereof reside on a single polypeptide chain.
28. The conjugate of claim 27 wherein said analyte is carbonic anhydrase III.
- 10 29. The conjugate of claim 28 depicted in SEQ ID NO:1.
30. A polynucleotide encoding the conjugate of claim 27.
31. A polynucleotide encoding the conjugate of claim 28.
- 15 32. A polynucleotide encoding the conjugate of claim 29.
33. A polynucleotide of claim 32 as set forth in SEQ ID NO:2.
- 20 34. A conjugate comprising an antibody to a first analyte or binding fragment thereof and a second analyte or fragment thereof, wherein independently, said antibody to said first analyte or binding fragment thereof in said conjugate is capable of binding said first

analyte, and said second analyte or fragment thereof in said conjugate is capable of being bound by an antibody to said second analyte.

35. The conjugate of claim 34 wherein said second analyte or fragment thereof is a protein or peptide.

36. The conjugate of claim 35 wherein said second analyte or fragment thereof and a heavy chain or light chain of said antibody reside on a single polypeptide chain.

37. The conjugate of claim 34 wherein said second analyte is carbonic anhydrase III and said first analyte is myoglobin.

38. A polynucleotide encoding the conjugate of claim 34.

39. A kit for identifying in a sample the presence or level of a first analyte over that of a second analyte comprising a labeling means adapted to label the first analyte through a binding interaction that is inhibited by the second analyte, and directions for use of said kit.

40. The kit of claim 39 wherein said labeling means comprises a conjugate having a component for binding the first analyte, and a component which binds antibody to the second analyte.

41. The kit of claim 39 wherein the labeling means comprises a second analyte and antibody to the first analyte.

42. The kit of claim 41, wherein the labeling means further comprises antibody to the second analyte having a detectable label bound thereto.

43. A kit for identifying in a sample the presence or level of a first analyte over that of a second analyte comprising:

- (1) a mobile, labeled binding partner to one of the first analyte or the second analyte,
- (2) a conjugate between the second analyte and a binding partner to the first analyte;
- (3) an immobilized binding partner to the other of the first analyte and the second analyte; and
- (4) instructions for use of said kit.

44. The kit of claim 43 wherein said binding partners are antibodies.

45. The kit of claim 44 wherein said second analyte is a peptide or polypeptide and said conjugate between the second analyte and an antibody to the first analyte comprises a single-chain polypeptide comprising said second analyte and a heavy chain or light chain of said antibody.

46. A kit for identifying in a sample the presence or level of a first analyte over that of a second analyte comprising the components of

- 1) a mobile, labeled binding partner to one of the first analyte or the second analyte,
- (2) a conjugate between the second analyte and one of biotin and streptavidin, and
- (3) a binding partner to the first analyte conjugated to the other of biotin and streptavidin;
- 5 (4) an immobilized binding partner to the other of the first analyte and the second analyte; and
- (5) instructions for use of said kit.

47. The kit of claim 46 wherein said second analyte is a peptide or polypeptide, said
10 conjugate between the second analyte and one of biotin and streptavidin is a single-chain polypeptide comprising said second analyte or epitope thereof and streptavidin or a biotin-binding fragment thereof, and said binding partner to the first analyte conjugated to the other of biotin and streptavidin is a binding partner to the first analyte conjugated to biotin.

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